Surveillance and Control of the *Anopheles gambiae* complex, Primary Malaria Vector in Liberia

by Peter J Obenauer and Craig A Stoops

Malaria is endemic in over 100 tropical and temperate countries and responsible for an estimated 300 to 500 million infections with over 1 million deaths worldwide, the majority being children under the age of five (Keiser et al, 2004; Collins and Paskewitz, 1995). The greatest malaria impacted region is Sub-Saharan Africa (SSA), which accounts for almost 90% of the malaria infections in the world (Collins and Paskewitz, 1995). While poverty is a major contributing factor for sustaining malaria transmission in the SSA region, malaria remains resilient largely because of one of the most efficient mosquito vectors in the world, the *Anopheles gambiae* complex. This group of mosquitoes is comprised of *An gambiae, An arabiensis, An merus, An melas* and *An quadriannulatus*. Each species within the complex is unique and has a specific larval habitat, making larval control efforts difficult. Compared to other malaria vectors, *An gambiae* is relatively long-lived, highly anthropophilic, has a short larval development period, and uses larval habitats created by human activity, traits that make this species ideal for vectoring malaria (Sinka et al, 2010). A second major vector in SSA is *An funestus*. It too, is extremely anthropophilic, but prefers semi-permanent bodies of waters containing emergent vegetation for larval habitats compared to *An gambiae* which prefers temporary sunlit pools. With the exception of the *An puctulatus* complex in New Guinea, few other places on earth contain the number of efficient malaria vectors as SSA (Collins and Pakewitz, 1995).

Liberia is located on the western coast of Africa and borders Guinea, Sierra Leone, and the Ivory Coast. US involvement in Liberia started in 1822 when freed US slaves were repatriated to eventually form a self-sustaining Republic by 1847. Over the past twenty years, Liberia experienced two consecutive civil wars (1989 – 1996 and 1999 – 2003) that resulted in an estimated 300,000 deaths and over 1.5 million displaced civilians. Peace was restored in the summer of 2003 when the United Nations removed President Charles Taylor from power. In response to the civil unrest and to provide additional security for the US Embassy, 225 Marines from the 26th Marine Expeditionary arrived in August 2003 at Roberts International Airport outside of Monrovia. After spending ten nights ashore, 80 Marines experienced symptoms of malaria, with 44 having to be medically evacuated from Liberia, of which 5 were diagnosed with complicated malaria including 2 with cerebral malaria and 3 with acute respiratory distress syndrome (Whitman et al, 2010). This attack rate

Figure 1: Dr Jeff Villinski poses with members from the Liberian Institute for Biomedical Research after they received certificates from a one week mosquito workshop held at the US Naval Medical Research Unit 3, Vector Biology Research Program, Cairo, Egypt.

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marks the largest malaria infection outbreak among US forces since Operation Restore Hope in Somalia, when 48 confirmed cases occurred in theater from December 1992 to May 1993; and over 200 cases once personnel returned to the United States (Wallace et al, 1996).

In 2007, President George Bush created the US Africa Command (AFRICOM) to provide military security and humanitarian operations to assist African nations in succeeding to become more economic and socially stable governments. In 2010, there were 113 diagnosed malaria infections among US military members; a higher rate compared to the six out of the eight previous years, with the majority attributed to *Plasmodium falciparum*. This represented the greatest attack rate since the 2003 Liberia outbreak (Anonymous, 2011). From 2000 – 2005, there were 423 malaria cases reported among the US military, 90% of them occurred in the Army and Marine Corps (Ciminera and Brundage, 2007). The majority of these cases occurred in Korea and Afghanistan, where *P vivax* is the predominant malaria parasite species. However, unlike *P vivax* which is rarely fatal, *P falciparum* is highly virulent, causes severe health complications and is responsible for 90% of the malaria infections in Liberia. Given increased US military engagements in Africa, future malaria infections are likely to increase without proper control measures. In fact, it has been determined that malaria infection could approach 100% if troops deployed to SSA were left unprotected (Smith and Hooper, 2005).

In December 2009, tragedy occurred in Liberia when a US Navy Seabee succumbed to cerebral malaria and while participating in Operation Onward Liberty (OOL). The goal of this joint military operation is to train and develop the Armed Forces of Liberia to be self-sufficient and capable of providing for their own internal defense. The symptoms and severity of malaria can vary greatly, depending on parasite species and the age of the mosquito. Clinical presentation can range from non-specific flu-like symptoms, especially early in the infection, to fever with chills, shakes, vomiting, diarrhea, headache, etc. However, without proper protection, infection with *P falciparum* can quickly lead to life-threatening complications, especially among naive individuals, which can include renal failure, bleeding, pulmonary edema, circulatory

Figure 2: Left - Members from the Navy Entomology Center of Excellence and the US Naval Medical Research Unit No. 3 prepare to apply insecticides; Photo by Mahmoud Saleh. Right - Hospital Corpsmen First Class Leslye Brown-Ruiz applies residual insecticides to the wall surface, Monrovia, Liberia; Photo by Darnell Gardner.

collapse, jaundice, seizures and death.

Seven additional malaria cases (all *P falciparum*) were reported among OOL members from January to March 2010. In response, AFRICOM requested the US Naval Medical Research No 3 (NAMRU-3) to investigate the outbreak among the approximately 62 US forces stationed throughout three camps in Montserrado County. The investigation revealed a lack of personal protection measures. Moreover, vector surveillance and control measures were nonexistent in the general vicinity of the bases.

In August 2010, NAMRU-3 was awarded a grant from the Global Emerging Infections System (GEIS) to conduct a study to investigate the distribution of the *An gambiae* complex and develop laboratory capacity for the Liberian Institute of Biomedical Research (LIBR). The work consisted of training technicians on mosquito colony maintenance, molecular techniques, vector identification and surveillance practices. Mosquito workshops for LIBR technicians have been held at NAMRU-3 to disseminate the latest information on vector surveillance, ELISA and molecular PCR diagnostics; see Figure 1. Another aspect of the study was to develop and monitor *An gambiae* populations for insecticide resistance while providing vector control for members of OOL. Three scientific objectives of the study were: 1) determine the distribution of the *An gambiae* complex, including the M and S forms through molecular techniques; 2) develop predictive models of *Anopheles* spp using geographical information system (GIS); and 3) determine the malaria infection rate among *Anopheles* spp and subsequently generate malaria risk maps based on those results.

To provide mosquito surveillance and reduce the number of malaria cases among members of OOL, NAMRU-3 collaborated with the Navy Entomology Center of Excellence (NECE), a subordinate command of the Navy and Marine Corps Public Health Center and a recognized Department of Defense asset committed to providing technical services and training to reduce the incidence of vector-borne diseases within the armed services.

Currently, we have established five mosquito surveillance sites throughout Liberia, three of which are on Liberian military installations. Every six months, inside walls of buildings that house US personnel are treated with residual insecticides (cypermethrin, lamda-cyhalothrin) dispersed from Stihl™ backpack sprayers; see Figure 2. Our success has been measured by the number OOL members reporting a considerable drop in the number of night-time mosquito bites, and since its inception in January 2010, no malaria cases have been reported. We credit some of this success to our spray operation. However, there is no substitute for strict anti-malaria discipline by employing direct observed therapy of chemoprophylaxis, consistently using treated bed nets, applying DEET regularly to the skin and wearing permethrin-treated clothing. Furthermore, command leadership is

Figure 3: CDR Pete Obenauer assists technicians from the Liberian Institute for Biomedical Research (LIBR) in placing mosquito traps baited with lures for attracting *Anopheles gambiae* at LIBR in Monrovia, Liberia. Photo by Mahmoud Saleh.

essential to stress the seriousness of employing these preventive measures.

Determining which member of a sibling species complex is responsible for the highest number of malaria cases infections is challenging, but essential for understanding malaria transmission dynamics. Members of the *An gambiae* complex and *An funestus* are two of the most efficient malaria vectors in Liberia and across Africa. Within the *An gambiae* complex, there are a number of biological differences between sibling species. For example, *An gambiae senu stricto* is a highly anthropophilic, endophilic species preferring temporary, sunlit, clear water pools as larval habitats compared to *An arabiensis* which is a zoophilic, exophilic, species and utilizes a variety of larval habitats including partially shaded streams to small natural and artificial containers (Sinka et al, 2010). To determine distribution, numerous adult mosquito traps baited with specific cues for the *An gambiae* complex have been placed throughout four sites in Liberia; see Figure 3. In addition, mosquito larvae are collected from multiple locations between sites in an effort to increase the number of sampling points. *Anopheles gambiae* complex larvae have been found in rice fields, sunlit pools, tire ruts, and even in artificial containers; see Figures 4 and 5. Collected mosquitoes are sorted and counted at LIBR and then processed and identified at NAMRU-3, using molecular techniques to determine the species complex. This information will be extremely useful when developing mosquito larval control measures, as M forms have adapted to more semi-permanent larval habitats such as rice fields, while S forms prefer temporary sites, such as rain puddles (Sinka et al, 2010).

Figure 4: *Anopheles gambiae* larvae have been found in different habitats, including rice fields and even in artificial containers. Photos by P J Obenauer.

Depending on future funding, the study and control program will continue through 2012. To date, over 12,000 mosquitoes have been collected and over 58 species have been identified. The data from our trap comparisons is currently being analyzed. The information gained will not only help protect US troops deployed to Liberia but will also provide valuable information for the US President's Malaria Initiative in Liberia and ultimately help the Liberian Ministry of Health control malaria, thereby helping all the people of Liberia.

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REFERENCES CITED

Anonymous. 2011. Update: malaria, US Armed Forces, 2010. MSMR. 18: 2-6.

Ciminera P and J Brundage. 2007. Malaria in the US military forces: a description of deployment exposures from 2003 through 2005. Am J Trop Med Hyg 76: 275-279.

Collins FH and SM Paskewitz. 1995. Malaria: current and future prospects for control. Annu Rev Entomol 40: 195-219.

Keiser J, J Utzinger, MC DeCastro, TS Smith, M Tanner, BH Singer. 2004. Urbanization in sub-saharan Africa and implication for malaria control. Am J Trop Med Hyg 71: 118-127.

Sinka ME, MJ Bangs, S Manguin, M Coetzee, CM Mbogo, J Hemingway, AP Patil, WH Temperely, PW Gething, CW Kabaria, RM Okaara, TV Boeckel, HCJ Godfray, RE Harbach, SI Hay. 2010. The dominant *Anopheles* vector of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic précis. Parasit Vector. 3: 1-34.

Smith AM and C Hooper. 2005. The mosquito can be more dangerous than the mortar round. Naval War College Review. 55: 77-97.

Wallace MR, TW Sharp, B Smoak, C Iriye, P Rozmajzi, SA Thornton, R Batchelor, AJ Magill, HO Lobel, CF Longer, JP Burans. 1996. Malaria among United States troops in Somalia. Am J Med 100: 49-55.

Whitman TJ, PE Coyne, AJ Magill, DL Blazes, MD Green, WK Milhous, TH Burgess, D. Freilich, SA Tasker, RG Azar, TP Endy, CD Clagett, GA Deye, GD Shanks, GJ Martin. 2010. An outbreak of *Plasmodium falciparum* malaria in US Marines deployed to Liberia. Am J Trop Med. Hyg. 83: 258-265.

Figure 5: *Anopheles gambiae* larvae have been found in different habitats, including rain-filled pools. Photos by P J Obenauer.

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